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Toxicity and cytotoxicity of the insecticide imidacloprid in the midgut of the predatory bug, *Podisus nigrispinus*

Luis Carlos Martínez[a,*], Angelica Plata-Rueda[b], Wagner Gonzaga Gonçalves[a], André Filipe Penha Aires Freire[c], José Cola Zanuncio[d], Hakan Bozdoğan[e], José Eduardo Serrão[a]

[a] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000 Viçosa, Minas Gerais, Brazil
[b] Instituto de Ciências Agrárias, Universidade Federal de Viçosa, 38810-000 Rio Paranaíba, Minas Gerais, Brazil
[c] Departamento de Ciências Biológicas, Universidade Federal de Maranhão, 65080-805 São Luís, Maranhão, Brazil
[d] Departamento de Entomologia, Universidade Federal de Viçosa, 36570-000 Viçosa, Minas Gerais, Brazil
[e] Ahi Evran University, TBMYO, 40100 Kirsehir, Turkey

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ABSTRACT

The selectivity of insecticides on natural enemies in pest control are an important strategy for Integrated Pest Management. However, insecticides can have side effects on non-target organisms such as natural enemies. This study evaluated the histological and cytological changes mediated by the sublethal concentration of the imidacloprid insecticide on the midgut of non-target predator *Podisus nigrispinus* (Heteroptera: Pentatomidae), used in the biological control of pests. Imidacloprid was toxic for *P. nigrispinus* with LC_{50} = 3.75 mg L^{-1} and survival of 51.8%. This sublethal concentration of imidacloprid causes histological alterations in the midgut epithelium and cytotoxic features were irregular border epithelium, cytoplasmic vacuolation, and apocrine secretions in the first 6 h after exposure with the insecticide. Apoptosis in the digestive cells occurs after 12 h of exposure in the midgut. These results suggest that imidacloprid may affect the digestive physiology of *P. nigrispinus* and compromise the effective predation of this insect a biological control agent. The associated use of this insecticide with the predator in pest control should be carefully evaluated.

1. Introduction

Podisus nigrispinus Dallas (Heteroptera: Pentatomidae) is a predatory bug used as a biological control agent to control of beetles and caterpillar pest in agricultural and forest plantations (Ferreira et al., 2008; Martínez et al., 2016). Regarding *P. nigrispinus*, several studies have detailed the development, histology and ultrastructure (Martínez et al., 2014a, 2016, 2017), predator-prey interaction (Ferreira et al., 2008), and biochemical process (Fialho et al., 2012).

Predators have tolerance relative to insecticides being used in Integrated Pest Management programs (IPM) (Kim et al., 2006; Cordeiro et al., 2010; Zanuncio et al., 2011). Chemical control is the common strategy for pest insects and its use has increased in several crops worldwide (Song and Swinton, 2009; Meissle et al., 2010; Pedlowski et al., 2012); However, the selectivity of insecticides to non-target organisms is important for IPM (Metcalf, 1980; Hardin et al., 1995; Desneux et al., 2007). In this sense, the search for safe insecticides for human health and the environment has resulted in the development of specific compounds for pests and selective to non-target insects (Matsumura, 2004; Nicholson, 2007; Biondi et al., 2012).

Various insecticides cause toxic effects but not exclusively resulting in insect death (Desneux et al., 2007). These effects may be physiological and related to development, longevity, fecundity, and behavior (Desneux et al., 2004; Kim et al., 2006; He et al., 2012). Imidacloprid is used in the control of many insect pests and has moderate toxicity to vertebrates (Horowitz et al., 1998; Boina et al., 2009; Martínez et al., 2014b).

Although the site of action of imidacloprid is the nervous system, other insect organs may be secondary targets (Catae et al., 2014, 2018; Fernandes et al., 2015). Among the non-target organisms of the insecticides, the medium intestine has been reported to be one of the most affected by these chemicals (Gutiérrez et al., 2016; Catae et al., 2018; Fiaz et al., 2018a). The midgut of predatory bugs (Pentatomidae) is anatomically divided into three regions: anterior, middle and posterior,

* Correspondence to: Departamento de Biologia Geral, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, Campus Universitário, 36570-000 Viçosa, Minas Gerais, Brazil.
E-mail addresses: lc.martinez@outlook.com (L.C. Martínez), angelicaplata@yahoo.com.mx (A. Plata-Rueda), wagner.gonzaga@ufv.br (W.G. Gonçalves), andre-aires2@hotmail.com (A.F.P.A. Freire), zanuncio@ufv.br (J.C. Zanuncio), hakan.bozdogan@ahievran.edu.tr (H. Bozdoğan), jeserrao@ufv.br (J.E. Serrão).

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which perform different functions in digestion (Fialho et al., 2012, 2013).

Data on the histological and cytological effects caused by the insecticide imidacloprid on the midgut of predatory bugs are scarce. We evaluated the acute toxicity and the histological and cytological changes in the midgut of *P. nigrispinus* mediated by the imidacloprid.

2. Materials and methods

2.1. Insects

Individuals of *P. nigrispinus* and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) were obtained from the mass establishment of the laboratory of Biological Control (Universidade Federal de Viçosa, Minas Gerais, Brazil). Adults of *P. nigrispinus* were maintained at 28 ± 2 °C, 80 ± 5% RH, 12:12 h (L: D) photoperiod and fed with *Tenebrio molitor* pupae and *Eucalyptus grandis* leaves. Pupae of *T. molitor* were kept in plastic trays (60 cm long × 40 cm wide × 12 cm high) with a temperature of 25 ± 1 °C, relative humidity of 70 ± 10% and 12:12 h (L: D) photoperiod. Adults of *P. nigrispinus* and *T. molitor* pupae, without apparent amputations or malformations, were used in the bioassays.

2.2. Toxicity test

Imidacloprid insecticide (Evidence® WG, Bayer, São Paulo, Brazil) was used in the acute toxicity tests and diluted in 1 L of water to produce stock solution, adjusting 100 g L^{-1} of insecticide to the required concentrations. The insecticidal efficacy was determined by calculating the lethal concentration values (LC_{25}, LC_{50}, LC_{75} and LC_{90}) under laboratory conditions. Six concentrations of imidacloprid in addition to the control (distilled water) were adjusted in 1 mL stock solution (treatments and distilled water): 0.312, 0.625, 0.125, 0.25, 0.5 and 10 mg L^{-1} (w/v). Pupae of *T. molitor* were soaked for 5 s at each concentration and allowed to dry in the environment. In the treatments, *T. molitor* pupae exposed to the insecticide was offered as food for an adult of *P. nigrispinus* in a glass vial (2 × 10 cm). Fifty adults of *P. nigrispinus* were used by concentration and the number of dead insects was counted after feeding with *T. molitor* pupae exposed to the insecticide up to 72 h. The lethal concentrations (LC_{25}, LC_{50}, LC_{75} and LC_{90}) and confidence limits were determined by regression based on the probit-mortality concentration (Finney, 1964) with PROC PROBIT procedure of SAS User v. 9.0 for Windows (SAS Institute, 2002).

2.3. Survivorship

Newly emerged adults of *P. nigrispinus* were fed with *T. molitor* pupae exposed to four concentrations of imidacloprid (LC_{25}, LC_{50}, LC_{75} and LC_{90}) as determined by the toxicity bioassay and control with distilled water. In survival test, insecticide exposure procedures were similar as described for the toxicity bioassays. Dead insects were quantified every six hours by 72 h. The data were submitted to survival analysis using the Kaplan-Meier estimator (Log-rank method) via the Origin Pro v 9.1 software (Originlab Corporation, 2013). Survival adults registered until the end of the experiment were treated as censored data.

2.4. Light microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration LC_{50} of imidacloprid at different time periods (30 min, 1, 3 and 6 h) and cryoanesthetized at − 4 °C. The midgut was dissected in insect saline solution (0.1 M NaCl + 0.1 M KH_2PO_4 + 0.1 M Na_2HPO_4), divided into the anterior, middle and posterior regions, and transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 12 h at 5 °C. The samples were then dehydrated in a grade ethanol series (70°, 80°, 90° and 95°) and embedded in historesin (Leica Biosystem Nussloch GmbH, Wetzlar, Germany). Sections 3 μm thick were obtained, stained with hematoxylin and eosin, and analyzed under an Olympus BX-60 light microscope (Olympus Corporation, Tokyo, Japan).

2.5. Transmission electron microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration LC_{50} of imidacloprid for 6 h and cryosanesthetized at − 4 °C. The midgut of *P. nigrispinus* (divided in anterior, middle and posterior region) were dissected and transferred to 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) containing 0.2 M of sucrose for 6 h at room temperature. The samples were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, followed by washing in the buffer and dehydrating in a grade ethanol series (70°, 80°, 90° and 99°). The samples were embedded in LR white resin (London Resin Company Ltd.) and ultra-thin sections (80–90 nm thick), obtained with PowerTomes PT-X ultramicrotome glass razor (RMC Boeckeler Instruments Inc., Tucson, AZ, USA), were compared with 1% aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined on Zeiss EM 109 transmission electron microscope (Carl Zeiss, Jena, Germany).

2.6. Immunofluorescence

The three regions of the midgut of *P. nigrispinus* exposed by 12, 24 and 36 h to estimated lethal concentration LC_{50} of imidacloprid were dissected in 0.1 M sodium phosphate buffer (PBS) and transferred to Zamboni's fixative solution for 2 h. Next, the samples were washed with PBS containing 1% Triton X-100 (PBST) and incubated with 1.5% bovine serum albumin in PBST for 2 h. The samples were incubated with anti-cleaved caspase 3 antibody (Cell Signaling Technology, Danvers, MA, USA) at 1:500 in PBS for three days at − 4 °C. After incubation, the samples were washed in PBS and incubated with anti-rabbit IgG secondary antibody, fluorescein isotiosinate (FITC) conjugated (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:500 in PBS for 24 h in the dark at − 4 °C. Midgut or *P. nigrispinus* were washed, and the cell nuclei were stained with TO-PRO-3 propidium iodide (Life Technologies, Carlsbad, CA, USA) for 1 h. Midgut were mounted on 50% sucrose glass slides and examined on Zeiss LSM510 META (Carl Zeiss, Jena, Germany) laser scanning confocal microscope.

3. Results

3.1. Toxicity and survivorship

The lethal concentrations of imidacloprid estimated by Probit (X^2 = 21.43; df = 5; P < 0.001) for *P. nigrispinus* fed with *T. molitor* pupae exposed to the insecticide were LC_{25} = 2.90, LC_{50} = 3.75, LC_{75} = 4.85 and LC_{90} = 6.27 mg L^{-1}. (Table 1, Fig. 1A). Mortality at the control was < 1%.

The survivorship of *P. nigrispinus* fed with *T. molitor* pupae exposed to imidacloprid showed differences between the concentrations (Log-

Table 1
Lethal concentration of the insecticide imidacloprid in *Podisus nigrispinus* for 72 h after feeding with pupae of *Tenebrio molitor*. Insecticide concentrations were applied using a topical solution on the prey. X^2, chi-square for lethal concentration and fiducial limits based on a logarithmic scale with significance level at $P < 0.0001$.

Concentration (df = 5)	Estimated values (mg L^{-1})	Fiducial limits		X^2
		Inferior	Superior	
LC_{25}	2.90	2.02	3.45	21.43
LC_{50}	3.75	3.05	4.41	
LC_{75}	4.85	4.14	6.27	
LC_{90}	6.27	5.16	9.69	

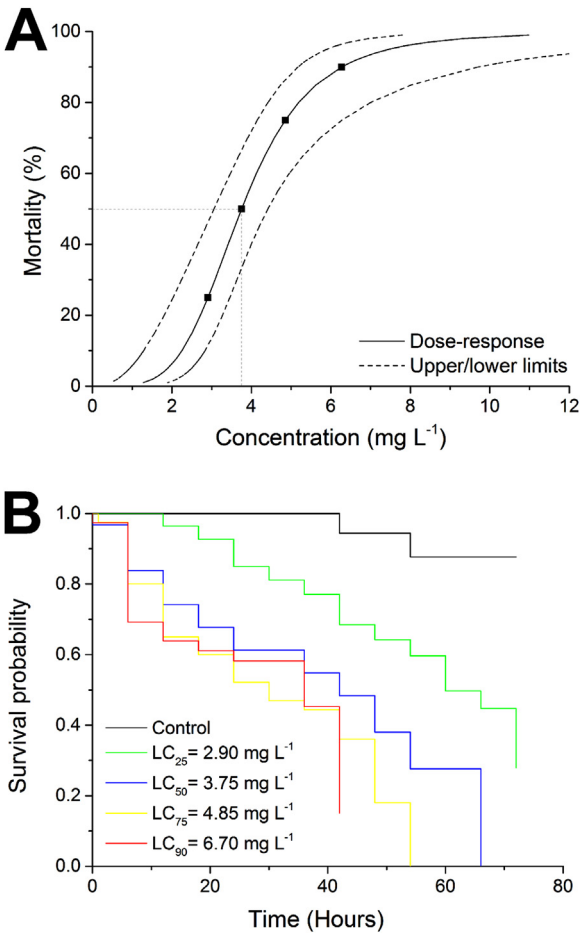

Fig. 1. Toxic effects of imidacloprid on *Podisus nigrispinus* fed on contamined prey. (A) Adult mortality caused by imidacloprid at different lethal concentrations (LC_{25}, LC_{50}, LC_{75} and LC_{90}) ($X^2 = 21.43$, df = 5, $P < 0.001$). Dotted lines denote 95% confidence intervals. Black point represents the LC_{50} concentration selected to evaluate morphological changes. (B) Survival curves of adults fed on prey exposed to different concentrations of imidacloprid for 72 h using the Kaplan-Meier method and compared using the log-rank test ($X^2 = 54.84$; $P < 0.001$).

rank test, $X^2 = 54.84$, df = 4, $P < 0.001$) (Fig. 1B). Survival was greater than 97.3% in the control adults after 72 h, decreasing to 51.8% with LC_{25}, 47.5% with LC_{50}, 29.9% with LC_{75} and 2.3% with LC_{90}.

3.2. Histopathology

The anterior midgut (AMG) of *P. nigrispinus* showed an epithelium with columnar cells in the control (Fig. 2A). The apical portion had striated border and the epithelial cells presented cytoplasm with little stained vesicles, small colored granules and nucleus with condensed chromatin. In the insects fed pupae exposed to imidacloprid LC_{50}, after 30 min, there was an increase in the number of vacuoles in the cytoplasm and apocrine secretion in the AMG lumen (Fig. 2B). One hour after feeding, the epithelium presented irregular apical surface, with intense vacuolization in the cytoplasm (Fig. 2C). The nucleus varied from spherical to elongated, occupying the medial-basal portion of the cells and with decondensed chromatin (Fig. 2C). Three hours after feeding of *P. nigrispinus* with pupae exposed to the insecticide, the apical surface of the epithelium was irregular with projections of the cytoplasm towards the lumen and increased apocrine secretion (Fig. 2D). These characteristics were observed up to 6 h after feeding (Fig. 2E). However, cytoplasmic vacuolation decreased 3 and 6 h after feeding pupae exposed to imidacloprid (Fig. 2D-E).

In the control, the middle-midgut (MMG) of *P. nigrispinus* showed an epithelium with columnar cells and the apical portion with a striated border (Fig. 2F). These epithelial cells presented cytoplasm with vesicles and spherical nuclei. After 30 min of feeding with pupae exposed to imidacloprid, the apical surface of the epithelium was irregular with high vacuolization in the cytoplasm apocrine secretion. The nucleus was elongated in the basal portion of the epithelium with decondensed chromatin (Fig. 2G). Vacuolization and cytoplasmic vacuole size decreased 1 h and 3 h after feeding, but apocrine secretion still occurred (Fig. 2H-I). Six hours after feeding with contaminated pupae, MMG was similar to the control (Fig. 2J).

The posterior midgut (PMG) showed an epithelium composed of high columnar cells, cytoplasm with vacuoles and high density of granules and vesicles, and the nucleus with condensed chromatin in the control insects (Fig. 2K). The epithelium presented an irregular apical surface with an increase in the number of vacuoles in the cytoplasm after 30 min and 1 h of feeding with pupae exposed to imidacloprid LC_{50} (Fig. 2L-M). Three and six hours after feeding with pupae exposed to the insecticide, the PMG epithelium showed high vacuolization, apocrine secretion and irregular nuclei with decondensed chromatin (Fig. 2N-O).

3.3. Cytotoxicity

In the control, the digestive cells of the AMG of *P. nigrispinus* showed apical surface with short microvilli and perimicrovilar membrane (Fig. 3A). The apical cytoplasm was rich in electron-dense vesicles, lysosomes and mitochondria, while the basal portion of the cells showed mitochondria associated with folds of the plasma membrane (Fig. 3C). In AMG of *P. nigrispinus* fed pupae of *T. molitor* exposed to LC_{50} of imidacloprid, after 6 h, cells showed apical cytoplasm with vacuoles, autophagosomes and electron-dense granules, in addition to a number of vacuoles secreted into the lumen (Fig. 3B), whereas in the basal portion of the cells, the infolds of the plasma membrane were elongated, dilated and in greater quantity (Fig. 3D) than in the control.

In the predatory bugs not exposed to imidacloprid, MMG digestive cells had short microvilli with perimicrovilar membrane (Fig. 4A). The cytoplasm presented many mitochondria, electron-dense vesicles, and lysosomes (Fig. 4A). The basal portion of the digestive cells was rich in mitochondria, secretory vesicles, and the basal plasma membrane with few short infolds (Fig. 4C). In those insects fed pupae exposed to imidacloprid, the digestive cells had vacuoles, autophagosomes and few electron-dense granules, some vesicles were released into the lumen (Fig. 4B), and the basal portion showed more infolds of the plasma membrane that were long (Fig. 4D).

The PMG of *P. nigrispinus* control had digestive cells with short microvilli and perimicrovilar membrane (Fig. 5A). In apical portion, Cytoplasm contained many mitochondria and showed secretory vesicles and electron-dense granules (Fig. 5A), the basal portion had some glycogen islands, and the basal plasma membrane had elongated folds associated with mitochondria. In the insects exposed to imidacloprid LC_{50}, the PMG had digestive cells with many autophagosomes and granules in the apical portion, in addition to vesicles released in the lumen (Fig. 5B), while the basal portion showed autophagosomes and the basal plasma membrane with long infolds (Fig. 5D).

The nucleus has a decondensed chromatin with some clusters of condensed chromatin and evident nucleolus in all the insects analyzed.

Regenerative cells were found in the three regions of the midgut and organized into nests with developed nuclei and cytoplasm with few organelles in all the insects evaluated.

3.4. Immunofluorescence

In the insects of the control group, there were few digestive cells with a positive reaction to cleaved caspase-3 (Fig. 6A, D, G). However, in *P. nigrispinus* fed pupae of *T. molitor* exposed LC_{50} of imidacloprid

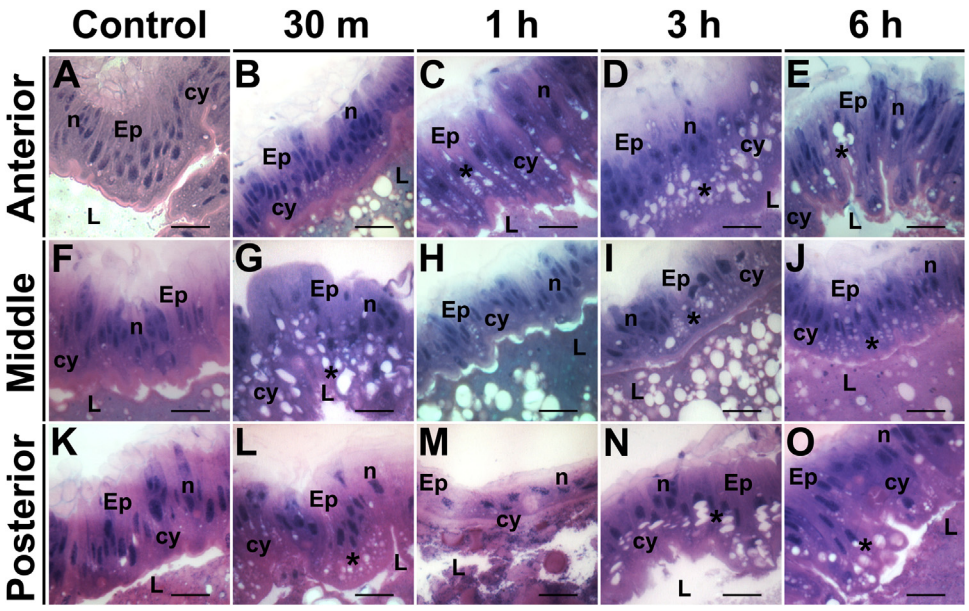

Fig. 2. Light micrographs of the midgut epithelium of *Podisus nigrispinus* after 30 min, 1, 3 and 6 h after feeding on prey exposed to imidacloprid. (A-E) Anterior midgut showing sequential effects with increase in vacuolization of digestive cells. (F-J) Middle midgut showing sequential effects with increase in vacuolization of digestive cells. (K-O) Posterior midgut showing sequential showing sequential effects with increase in vacuolization of digestive cells. Epithelium (*Ep*), lumen (*L*), cytoplasm (*cy*), nucleus (*n*); cytoplasmic vacuolization (*). Control (A, F, K). (Bars = 20 µm).

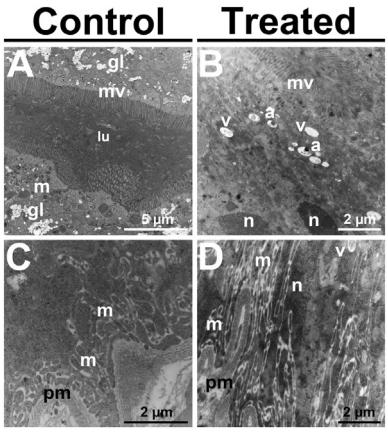

Fig. 3. Transmission electron micrographs of the digestive cells of the anterior midgut of *Podisus nigrispinus* fed on prey exposed to imidacloprid. Control (A and C) and insecticide exposure (B and D). (A) Digestive cells showing short microvilli (*mv*) associated with the perimicrovilar membrane in the cell apical portion, cytoplasm rich in mitochondria (*m*) and some glycogen islands (*gl*). Lumen (*lu*). (B) Insects exposed to the insecticide showing short microvilli (*mv*), well-develop nucleus (*n*), vacuoles (*v*) and autophagosomes (*a*) in the cell apical portion. (C) Note plasma membrane infolding (*pm*) short with numerous mitochondria (*m*) associated in cell basal portion. (D) Plasma membrane infoldings (*pm*) long and enlarged with well-develop nucleus (*n*), few mitochondria (*m*) and vacuoles presence (*v*) associated in the cell basal portion.

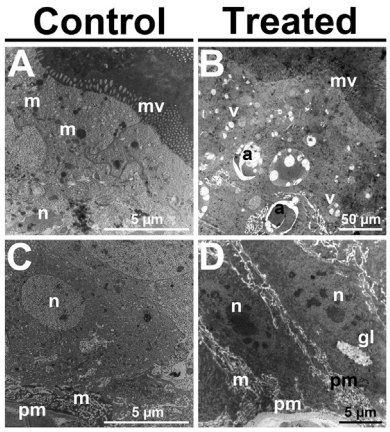

Fig. 4. Transmission electron micrographs of the digestive cells of the middle midgut of *Podisus nigrispinus* fed on prey exposed to imidacloprid. Control (A and C) and insecticide exposure (B and D). (A) Digestive cells showing short microvilli (*mv*) associated with perimicrovilar membrane, cytoplasm rich in mitochondria (*m*) and well-develop nucleus with decondensed chromatin in the cell apical portion. (B) Digestive cells exposed to the insecticide showing short microvilli (*mv*), cytoplasm with numerous vacuoles (*v*) and autophagosomes (*a*) in the cell apical portion. (C) Plasma membrane infolding (*pm*) short with well-develop nucleus (*n*) and numerous mitochondria (*m*) associated in cell basal portion. (D) Plasma membrane infoldings (*pm*) long and enlarged with well-develop nucleus (*n*), mitochondria (*m*), and glycogen island (*gl*) associated in cell basal portion.

after 24 and 36 h there was an increase in digestive cells with positive reaction to caspase-3 cleaved in the AMG (Fig. 6B-C), MMG (Fig. 6E-F) and PMG (Fig. 6H-I).

4. Discussion

The toxicity caused by the insecticide imidacloprid in the predatory bug, *P. nigrispinus* was determined and the histo-cytological changes were observed in the midgut. Imidacloprid is toxic to *P. nigrispinus* (LC_{50} = 3.75 mg L^{-1}) and reduces its survival when this predator was fed prey to the sublethal concentration LC_{25} (2.90 mg L^{-1}). These data show that the non-target predator, *P. nigrispinus* is susceptible to neonicotinoid insecticides. The susceptibility of the insects may vary with the exposure of the imidacloprid by ingestion (He et al., 2012; Martínez et al., 2014b; Fernandes et al., 2015; Catae et al., 2018). Predatory bugs such as *Dicyphus tamaninii* Wagner (Miridae), *Podisus maculiventris* (Say) (Pentatomidae) and *Orius laevigatus* Fieber (Anthocoridae) are sensitive to imidacloprid at different concentrations (De Cock et al., 1996; Figuls et al., 1999; Angeli et al., 2005). In *P. nigrispinus*, toxic effects and low survival have been reported after ingestion of prey contaminated with azadirachtin (Zanuncio et al., 2016), deltamethrin (De Castro et al., 2013), and thiametoxam (Torres et al., 2003). Imidacloprid is an insecticide with a systemic action that interferes with the transmission of nerve impulses in insects by irreversible and specific binding to nicotinic acetylcholine receptors (Buckingham et al., 1997; Simon-Delso et al., 2015). Specific binding causes the channel opening and depolarization of post-synaptic neurons, resulting in paralysis and death (Elbert et al., 2008). Despite being effective in controlling pest insects, our study suggests that imidacloprid is not selective for this non-target

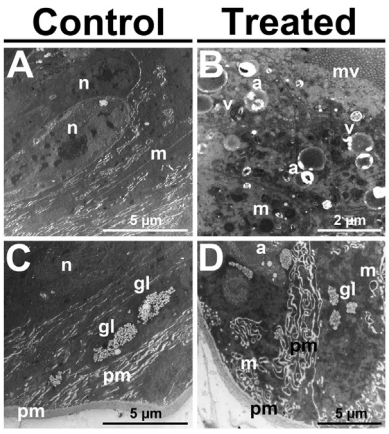

Fig. 5. Transmission electron micrographs of the digestive cells of the posterior midgut of *Podisus nigrispinus* fed on prey exposed to imidacloprid. Control (A and C) and insecticide exposure (B and D). (A) Digestive cells showing cytoplasm well-develop nucleus (*n*) and cytoplasm with mitochondria (*m*) associated in cell apical portion. (B) Digestive cells exposed to the insecticide showing short microvilli (*mv*), cytoplasm with numerous mitochondria (*m*) vacuoles (*v*), and autophagosomes (*a*) in the cell apical portion. (C) Plasma membrane infoldings (*pm*) long and enlarged with well-develop nucleus (*n*), and glycogen island (*gl*) associated in cell basal portion. (D) Plasma membrane infoldings (*pm*) long and coiled threads with glycogen island (*gl*) and numerous autophagosomes (*a*) associated in cell basal portion.

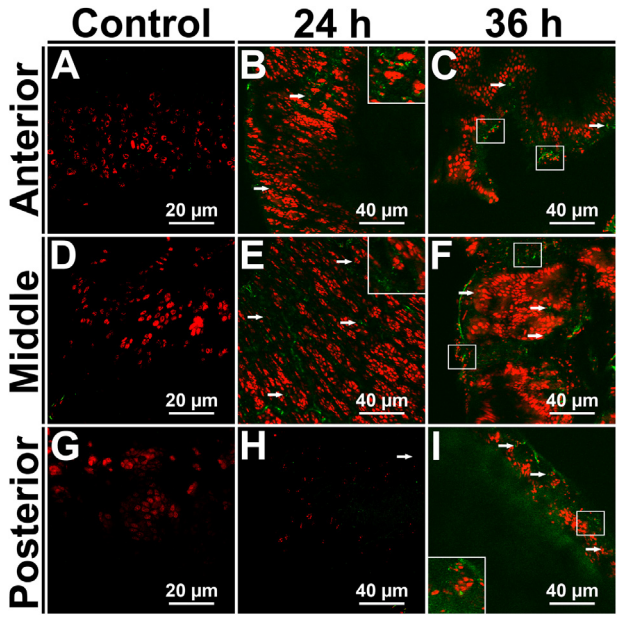

Fig. 6. Immunofluorescence for detection apoptosis in the midgut fo *Podisus nigrispinus* 24 and 36 h after feeding on prey exposed to imidacloprid. (A-C) Anterior midgut. (D-F) Middle midgut, showing active cleaved caspase-3 (*arrows*) and some regions with high activity of caspase-3 (*white square*). (B, E, I) Increased image showing specific of active cleaved caspase-3.

insect used as pest control agents.

Still in sublethal doses, imidacloprid showed histopathological effects on the non-target organ, medium intestine of *P. nigrispinus* with features of cell degeneration, such as cytoplasmic vacuolization and chromatin decondensation. Cell degeneration in the midgut has been reported in non-target insects such as *Ceraeochrysa claveri* Navas (Neuroptera: Chrysopidae) exposed to azadirachthine (Scudeler and dos Santos, 2013), *Apis mellifera* L. (Hymenoptera: Apidae) in response to thiamethoxam (Catae et al., 2014) and *Calliabaetis radiatus* Navas (Ephemeroptera: Baetidae) to deltamethrin (Gutiérrez et al., 2016).

In *P. nigrispinus*, the effects of imidacloprid are more intense in the AMG and MMG between 30 min and 1 h after feeding the contaminated prey, with reduction of the cellular degeneration after 3 h, while in the PMG, the degenerative effects occur from 3 h and increase up to 6 h. A possible explanation for the first effects is the passage time of the insecticide along with the food in the midgut, but histopathological data suggest that the processes of digestion and absorption of food are compromised throughout the midgut.

Imidacloprid causes increased apocrine secretionin the midgut of *P. nigrispinus* during the first 6 h of feeding with contaminated prey, suggesting that the intestinal epithelial cells also undergo some detoxification processes, thus reducing the effects of the insecticide. The detoxification processes in the insect's midgut is not unexpected, since functions of this organ include the production of detoxification enzymes glycosyltransferases (GTSs), cytochrome P450s (P450s), and carboxylstases (SCCs) involved in the metabolism of insecticides (Feyereisen, 1999; Li et al., 2006; Meech et al., 2012). In the present study, it was observed that the vesicles were found in the cytoplasm (Ferreira et al., 1990; Aumüller et al., 1999), which was released by apocrine secretion in the lumen of the midgut (Ferreira et al., 2013). Thus, the increase of apocrine secretion in the midgut of *P. nigrispinus* fed with prey exposed to imidacloprid may be due to the release of enzymes to detoxification as observed in other insects (Yu and Hsu, 1993; Enayati et al., 2005; Zhu et al., 2011).

The cytotoxicity caused by imidacloprid was observed in the three regions of the midgut of *P. nigrispinus* with increased granules and vacuoles secreted into the lumen, autophagosomes and dilatation of the folds of the basal plasma membrane in the digestive cells. These characteristics correspond to degenerative cellular events also described in *A. mellifera* midgut epithelial cells exposed to fipronil (Cruz et al., 2010) and spinosade (Lopes et al., 2018).

In *P. nigrispinus*, cytotoxic effects started with AMG followed by MMG and PMG. The differences in cellular responses caused by imidacloprid in the different regions of the midgut are due to the movement of the contaminated food. AMG has more severe cellular degeneration probably because they come into contact with the insecticide-contaminated food in the first stages of digestion (Catae et al., 2014; Oliveira et al., 2014), compromising the digestion and absorption processes (Terra, 1990; Fialho et al., 2012; Torres and Boyd, 2009). Cytoplasmic vacuolation suggests that the cell is in the early stage of autophagic degradation of damaged cellular components in response to chemical/physiological stress (Clarke, 1990; Lockshin and Zakeri, 2004). Autophagy does not necessarily culminate in cell death, as it is a normal physiological process of recycling proteins and organelles (Ferreira et al., 2013; Rossi et al., 2013; Yoshimori, 2004). However, if chemical stress prevails, the cell may die from autophagy or trigger apoptosis (Lockshin and Zakeri, 2004; Rossi et al., 2013; Fiaz et al., 2018b). Thus, the cytotoxic effects caused by imidacloprid show that the digestive cells of *P. nigrispinus* can not maintain digestion processes and interrupt the nutrient absorption and results in high energy cost in the detoxification of the insecticide.

Imidacloprid induces a progressive increase of cell death in the midgut of *P. nigrispinus* as demonstrated by the cleaved caspase-3. Activation by caspase during apoptosis consists of permeabilization of the mitochondrial membrane, DNA fragmentation, leading to cell destruction (Green, 2005; Nikoletopoulou et al., 2013; Martínez et al., 2018). Morphological changes such as chromatin condensation, nucleus fragmentation and vacuolization are typical signs of apoptosis (Häcker, 2000; Ziegler and Groscurth, 2004; Rost-Roszkowska et al., 2008; Nikoletopoulou et al., 2013). Various clusters of condensed chromatin were distributed in the midgut of *P. nigrispinus*, contrasting with classical nuclei of apoptosis where condensation begins peripherally along the nuclear membrane (Häcker, 2000; Ziegler and Groscurth, 2004). This can be attributed to the existence of alternative models of programmed cell death that may result in less compact chromatin (Leist and Jäättelä, 2001; Broker, 2005; Martínez et al., 2018). Our results

suggest that imidacloprid induces cell death in the three regions of the midgut of *P. nigrispinus*, as demonstrated by the presence of cleaved caspase-3 in inmunoflorescence as for histopathological and cytotoxical test.

Our study shows that imidacloprid is toxic to the predatory bug *P. nigrispinus* via ingestion and reduces its survival between 48 and 72 h. When the insect was exposed to imidacloprid LC_{50}, cytoplasmic vacuolation, irregular border epithelium and widening of the space between the epithelium and the basement membrane indicated that digestive secretory cells are at the early stage of cell death in response to the chemical/physiological stress caused by the insecticide. The toxic and cytotoxic effects caused by imidacloprid may affect the nutrient uptake and disaggregation of *P. nigrispinus* indicating that the joint use of this insecticide and the predator should be evaluated during the management of agricultural and forest pests.

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